

Oxidative stress in endothelial cell dysfunction and thrombosis

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Key Words

redox chemistry, nitric oxide, platelets

Abstract

Endothelial dysfunction (ECD) is the earliest phenotypic change in the vasculature following exposure to atherothrombotic risk factors. ECD is associated with decreased synthesis and increased oxidative inactivation of nitric oxide (NO). Critical antioxidant enzymes essential for eliminating reactive oxygen species that can inactivate NO include the superoxide dismutases, the glutathione peroxidases, catalase, and glucose-6-phosphate dehydrogenase. Deficiencies of these enzymes increase oxidative stress and NO inactivation and, as such, can either lead to ECD or account for the underlying mechanism of ECD associated with a given atherothrombotic risk factor. Selected antioxidants improve intracellular redox state and reverse ECD by improving the bioavailability of NO. These observations provide mechanistic insights into the molecular basis of ECD in vascular disease and its treatment.

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Aerobic metabolism in mammals generates potentially damaging reactive oxygen species (ROS) against which pro-

TECTIVE antioxidant defenses have evolved. These ROS derive from several biochemical and enzymatic sources, including mitochondrial respiration, the oxidation of glucose by glucose oxidase, the oxidation of xanthine by xanthine oxidase, the nitric oxide synthases, and the NAD(P)H oxidases. Established atherosclerosis as well as risk factors for atherosclerosis lead to the generation of ROS and, thereby, to vascular oxidant stress and injury.

Endothelial dysfunction (ECD) is the earliest phenotypic change in the vasculature following exposure to atherothrombotic risk factors. Hypertension, hypercholesterolemia, cigarette smoking, diabetes mellitus, and hyperhomocysteinemia all induce ECD in the absence of established atherothrombotic disease [1,2]. ECD is manifest as a conversion of the normal endothelial phenotype from one that promotes smooth muscle relaxation, impairs platelet activation and fibrin formation, limits vascular permeability to blood cells and macromolecules, and prevents smooth muscle proliferation to one that supports an increase in smooth muscle tone, promotes thrombosis, adheres to circulating leukocytes, and permits smooth muscle proliferation.

A key molecular mediator of normal endothelial function is nitric oxide (NO). This simple heterodiatomic molecule is normally generated from the oxidation of L-arginine by endothelial NO synthase. In ECD, the bioavailability of NO is decreased, largely owing to an increase in ROS. Two principle mechanisms yield less bioactive NO in ECD. First, NAD(P)H oxidase activity is increased, leading to an increase in the generation of superoxide anion which reacts with NO to produce

peroxynitrite (OONO-) oxidatively inactivating the NO. Second, endothelial NO synthase itself undergoes "uncoupling," which means that this oxidoreductase is converted from an enzyme that oxidizes L-arginine to NO into one that reduces molecular oxygen to superoxide anion. This enzymatic uncoupling occurs with a decrease in cofactors required for NO synthesis, especially tetrahydrobiopterin, as well as a decrease in the availability of L-arginine owing to the synthesis of a competitive inhibitor present in the setting of ECD, viz., asymmetric dimethylarginine. Thus, ECD is associated with decreased synthesis and increased oxidative inactivation of NO.

Critical antioxidant enzymes essential for ridding the normal and dysfunctional endothelial cell of ROS include extracellular superoxide dismutase (EC-SOD) found on the external endothelial cell membrane, Cu,Zn SOD located in the cytoplasm, Mn SOD located in the mitochondria, catalase, and glutathione peroxidase-1 (GPx-1). The SODs dismutate superoxide anion into hydrogen peroxide, which is then reduced to water by catalase. Hydrogen peroxide and lipid peroxides are also reduced to their corresponding alcohols by GPx-1, which utilizes glutathione as an obligate cosubstrate. Genetic deficiencies of SODs or of GPx-1 promote oxidant stress in the vasculature [3], limiting nitric oxide availability. Importantly, hyperhomocysteinemic states are uniquely pro-oxidant owing to the selective transcriptional suppression of the GPx-1 gene by homocysteine [4]. Plasma GPx (GPx-3) has also recently been shown to have an important role in eliminating extracellular ROS in the vascular environment, thereby preventing

oxidative inactivation of endothelium- and platelet-derived NO [5,6]

Very recently, we have also demonstrated that glucose-6-phosphate dehydrogenase (G6PD) is a key antioxidant enzyme in vascular cells [7]. This enzyme is the rate-limiting enzyme in the pentose phosphate pathway, but is also the principal source of NADPH in the cytosol of endothelial and vascular smooth muscle cells. Inhibition or a deficiency of G6PD leads to decreased NADPH stores, which, in turn, decreases glutathione levels and glutathione disulfide reductase activity. This decrease in the glutathione pool impairs GPx-1 activity, leading to an increase in intracellular ROS and a decrease in bioavailable NO [7]. Importantly, a deficiency of NADPH uncouples NO synthase activity owing to a decrease in this essential cofactor and glutathione, as well as a decrease in tetrahydrobiopterin, the synthesis of which requires NADPH.

Antioxidants that maintain intracellular thiol redox state, such as L-2-oxothiazolidine-4- carboxylic acid [8], improve ECD and enhance the bioavailability of endothelial NO. These data suggest that well-designed, optimally partitioned antioxidant therapies have the potential to have both functional and clinical benefit in selected patient populations with atherothrombotic disease.

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